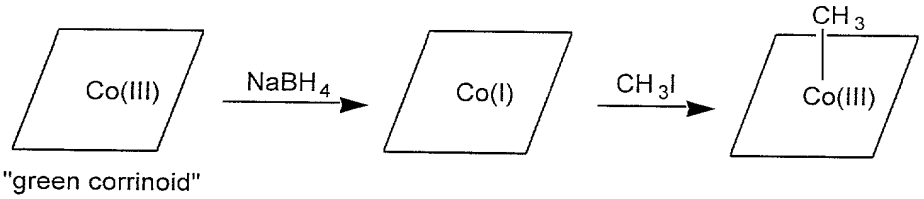
A bioconjugate containing the "green corrinoid" (Brown et al., 1996) can be synthesized as follows. The "green corrinoid" can be reduced in analogy to cobalamin, and that the reduced corrinoid will react with iodomethane to form the methylCo(III) corrinoid. Since the methylCo(III) corrinoid exhibits similar behavior to natural cobalamin, similar conjugation procedures with the electrophilic drug derivatives described above can be carried out.



"green corrinoid"

The bioconjugates of the present invention have the improved property of being capable of targeted, selective release of the bioactive agent from the bioconjugate. The bioactive agent is is released from the bioconjugate by cleavage. In one embodiment, the cleavage may occur as a result of normal displacement by cellular nucleophiles or enzymatic aciton. In a second embodiment, the cleavage is caused to occur selectively at the release site by an external signal. The external signal may be light or photoexcitation, i.e. photolysis, or it may be ultrasound, i.e. sonolysis. Further, if the photolysis takes place in the presence of a magnetic field surrounding the release site, the release of the drug, such as a cytotoxic agent, into surrounding healthy tissue can be minimized.

Although it is desired to cause the bioactive agent to be released at the desired cells, tissue or organs, e.g., at the site of the tumor or other cancer cells, it is also desirable to protect adjacent tissues from the negative side effects of such potent agents. The bioactive agent is released from the bioconjugate at the targeted site preferably by application of an external signal, such as light or ultrasound. The photolysis of the bioconjugates of the present invention occurs though cleavage of the Co-C bond to produce a solvent-caged radical pair consisting of Co(II) and the bioactive agent radical (R·). Lott et al. (1995) demonstrate that alkylcob(III)alamin photolysis can undergo magnetic field dependent recombination. A magnetic field application of 100-3000 gauss can be used to enhance radical pair recombination in surrounding tissue where drug release from the conjugate is not desired, leading up to at least a 2-fold decrease in photochemically-triggered drug release in such surrounding healthy tissue.

The sonolysis of the bioconjugates of the present invention occurs through cleavage of the Co-non-reactive atom bond in aqueous solution to produce the bioactive agent and a Co(II) (e.g., cob(II)alamin (Cbl^{II})) under anaerobic conditions or the bioactive agent and aquoCo-(III) (e.g., aquocob(III)alamin (H_2O-Cbl^{III})) under aerobic conditions. In either event, sonolysis from the focused application of ultrasound, results in Co-non-reactive atom bond cleavage and the irreversible release of the bioactive agent from the organocobalt complex.

The bioconjugates of the present invention can undergo natural cleavage as follows. Bioconjugates may be cleaved by natural means such as by serum nucleophiles. Once inside the cell, cobalamin-drug bioconjugates (utilized here only as an example and not intended to limit the invention) can undergo cleavage by a variety of mechanisms. Standard B_{12} ligand exchange mechanisms permit the displacement of the drug. Alternatively, cellular nucleophiles can attack at either the carbon or the cobalt atoms of the Co-C bond. Cyanide is the most common example of a nucleophile which attacks at cobalt, leading to cyanocob[III]alamin and a free drug in which the former C-Co bond has been replaced with a C-H bond. Thiols (such as are found in cysteine or glutathione) can attack at carbon, leading to a reduced cob[I]alamin and a free drug which incorporates the former thiol group. (e.g., R-Co[III] + R'-SH + base -> R-S-R' + Co[I] + base-H.) Hydroxide and other basic agents can also cleave organic ligands from Co[III] complexes, although this typically occurs via an elimination process which alters the structure of the ligand through incorporation of a new double bond. In addition, B_{12} metabolic enzymes present in cells can result in the cleavage of the bioactive agent from the co-atom.

The bioconjugates of the present invention can undergo cleavage by photoactivation or photolysis as follows. The photochemical release of the bioactive agent from the organocobalt complex can be triggered by the application of visible light at 400-800 nm. It is preferred to use organocobalt complexes which require longer wavelengths of visible light (600-800 nm), more preferably red light (600 to 750 nm). When photolysis is utilized for the release of the bioactive agent form the bioconjugate, it is particularly preferred that the non-reactive atom of the bioactive agent or the atom of the spacer bound to the cobalt atom be a carbon atom.

The vitamin B_{12} cofactor occurs naturally in two forms: adenosylcob(III)alamin, ($AdoCbl^{III}$), also known as coenzyme B_{12}, and methylcob(III)alamin (CH_3Cb^{III}). The remarkably weak C-Co bond from the corrin ring to the 5'-deoxyadenosyl or methyl ligand imparts a most unusual chemistry to cobalamins. The C-Co bond energy in $AdoCbl^{III}$ and CH_3Cbl^{III} is

estimated to be as low as 31 and 37 kcal/mole, respectively. This makes the C-Co bond one of the weakest covalent bonds known and allows photocleavage of the bond by visible light. The initial product of the photocleavage of $AdoCbl^{III}$ is the geminate radical pair $\{CH_2\text{-}Ado : Cbl^{II}\}$. Brackets { : } indicate the radical pair is *geminate* (born of the same parent molecule) and held in close proximity by solvent interactions. Picosecond laser flash photolysis experiments of $AdoCbl^{III}$ have shown this to be a reversible process with a geminate recombination rate constant of $k_{rec} \approx 1 \times 10^9\ s^{-1}$, following photolysis. Recent nanosecond laser flash photolysis studies have probed a slower radical pair recombination that occurs in the solvent and is limited by diffusion.

The π-π^* electronic transition of the corrin ring of cobalamin produces a long wavelength absorption maximum at 525 nm, as shown by Figure 1. Irradiation of alkylcobalamins at this wavelength leads to cleavage of the C-Co bond with a photolysis quantum yield of 0.1-0.3. The *in vivo* photolysis of bioconjugates according to the present invention is preferably accomplished by delivering the light with a fiber optic probe because of the strong absorption of hemoglobin near this wavelength. To avoid potential problems with the absorption spectrum of cobalamin while maintaining a photolabile C-Co bond, Co[SALEN], which is a five-coordinate analogue of coenzyme B_{12}, can be used. Alkyl-Co[SALEN] complexes have absorption maxima near 650 nm, with significant absorption beyond 700 nm as shown by Figure 2. This is a distinct advantage for photoactivatable drug release, as human tissue becomes increasingly transparent above 610 nm. Other synthetic or naturally occurring B_{12} derivatives are, or may become, available that have absorption maxima above 600 nm.. For example, a green cobalt corrinoid having an absorption maximum at about 624 nm is reported by Brown et al. (1996). Human tissue becomes transparent to depths of up to about 5.7 cm at wavelengths of between about 600 and 800 nm. The use of longer wavelengths enables the more selective irradiation of limited portions of a subject's body, with consquent release in a small target region.

The bioconjugates of the present invention can undergo cleavage by photoactivation or photolysis in the presence of a magnetic field as follows. The use of the magnetic field further limits the release of the bioactive agent to the desired site. For example, because of the toxicity of antineoplastic agents to healthy tissues, it is incumbent upon any effective site specific delivery system to limit damage to cells other than at the site of activity. In the photohomoylsis cleavage reaction, the electrons in the broken covalent bond start out with their spins paired ↑↓ (singlet state) from having participated in the covalent bond. During the early lifetime of the